6894

Preparation of Vitamin C from Lemon Juice.

W. A. WAUGH,* O. A. BESSEY* AND C. G. KING.

From the Department of Chemistry, University of Pittsburgh.

The isolation and identification of vitamin C[1] has stimulated research in many laboratories where the crystalline substance is desired. Our original procedure has been successfully followed in other laboratories, but the present detailed procedure can be followed more readily, with larger yields, and in less time. Working with one to 6 liters of lemon juice, consistent yields of 100 to 150 mg. per liter of the crystalline vitamin may be regularly obtained.

Filter 3 l. of juice through muslin, add 7 gm. of copper-free basic lead carbonate ($2PbCO_3.Pb(OH)_2$) per 100 cc., stir with an electric stirrer for 3 hr., add saturated neutral lead acetate solution (about 500 cc. per 3 l. preparation), continue stirring for 30 min., and centrifuge (ppt. is inactive, but may be rinsed for further recovery). Maintain a stream of CO_2 bubbling through the liquid while cooling to 0°C., add NH_4OH (1:3), with constant stirring, to the liquid until the pH reaches 7.6 (as shown by phenol red on a spot plate), centrifuge, discard the liquid (inactive), redissolve the yellow precipitate with acetic acid (1:3), and reprecipitate with NH_4OH at a pH of 7.6. (If either of the above liquid portions remain yellow, probably not enough lead acetate solution or ammonia was added). Dissolve the precipitate slowly with hydrochloric acid (1:1), adding acid until the solution will turn Congo red distinctly blue. Extract the entire preparation with one-half volume of n-butyl alcohol (to remove oils, etc.) and then add ethyl alcohol until the concentration is about 75%. Centrifuge (ppt. is mostly $PbCl_2$), evaporate to about 10 cc., add 100 cc. of acetone, centrifuge (ppt. mostly inorganic salts), add enough $BaCO_3$ to neutralize free HCl (to Congo red), and evaporate to dryness in the presence of fine white sand (about 5 gm.). Add 15 cc. of dry benzene and again evaporate to dryness. Cover and permit to extract over night with

* The authors are greatly indebted to Parke, Davis & Company, and The Abbott Laboratories, for a Research Fellowship Grant during the course of this investigation, and to the California Fruit Growers Exchange for supplying the lemons used.

[1] *Science*, 1932, **75**, 357; 630.; *J. Biol. Chem.*, 1932, **97**, 325; *Cf.*, *Ann. Repts. Soc. Chem. Ind.*, 1932, 547.

200 cc. of absolute acetone (the organic residue is largely inositet). Rinse, centrifuge, and evaporate the solution to dryness. Dissolve the residue in 30 cc. of absolute n-propyl alcohol, cool to 0°C. and add an equal volume of petroleum ether (B.P. 30-60°). Centrifuge off the precipitate and add to the solution 60 cc. more of the petroleum ether, keeping the solution near 0° (most of the vitamin remains in solution). Extract the 2 separate precipitates in turn with 25 cc. of n-propyl alcohol, for about 10 min., at room temp., cool, and again precipitate slowly by the addition of 75 cc. of petroleum ether. Centrifuge, rinse, and discard the precipitate (after testing for reducing action). Combine the 3 liquid portions and evaporate to dryness. Add 15 cc. of dry benzene and again evaporate completely. Redissolve in a minimum volume of n-propyl alcohol, cool, and add petroleum ether (B.P. 135-150°) until it begins to cloud slightly, and let stand in an open beaker in a desiccator over P_2O_5 in a refrigerator. If crystallization is not practically complete within 24 hr. evaporate the alcohol slowly in a vacuum desiccator. For recrystallization methyl alcohol may be used satisfactorily. The crystals may be rinsed with ice-cold glacial acetic acid or acetone.

Notes. The water used for preparing solutions should be redistilled from glass, and all reagents, especially the lead salts should be free from copper. The acetone, propyl alcohol, and petroleum ether should be carefully dried, or crystallization will not be satisfactory. An excess of HCl during acetone and subsequent evaporations will cause darkening and the formation of resinous material. If a second liquid phase appears during the first acetone separation, the aqueous phase should be further extracted or there will be a serious loss of the vitamin in the syrupy layer which occasionally forms at the bottom of the solution. Evaporations should be carried out under reduced pressure (below about 40°), with a capillary tube carrying a stream of CO_2. It is possible to check each step in the procedure by titrating small aliquot portions with acid iodine solution or with 2, 6-dichloro-phenol-indophenol,[2] in case any question arises concerning a loss in activity. In our experience titration with the dye has been satisfactory as an index of the vitamin C content of both plant and animal tissues, but the iodine titration is

† Identified by Dr. E. K. Nelson (private communication).

[2] Gibbs, Cohen and Cannon, *Public Health Reports*, 1925, **40**, 649; Tillmans, Hirsch and Hirsch, *Z. Untersuch. Lebensm.*, 1932, **63**, 1; Mottern, Nelson and Walker, *J. Assn. Off. Agr. Chem.*, 1932, 614; Birch and Dann, *Nature*, 1933, **131**, 469.

only satisfactory with purified material. Centrifuging precipitates instead of filtering facilitates protection from air and moisture, and working rapidly at lower temperatures.

6895

Amino Acids as Gastric Secretagogues.

GEORGE R. COWGILL AND ELIZABETH R. B. SMITH.*

From the Department of Physiological Chemistry, Yale University.

In the course of our recent studies on the secretion of gastric pepsin, we examined the effects of small amounts of amino-acids administered by the technique of Tagawa[1] to Pavlov gastric pouch dogs. The volumes of juice obtained were too small to allow accurate calculations of the total pepsin contents to be made, and the volume of secretion obtained differed greatly from that reported by Tagawa. In the present study 13 amino-acids were tested: alanine, arginine monohydrochloride, aspartic acid, cystine, glutamic acid, glutamic acid hydrochloride, glycine, histidine monohydrochloride, leucine, isoleucine, phenylalanine, tyrosine and valine.

As has been emphasized by Ivy and Javois,[2] all of the amino-acids appeared to be rather weak secretagogues with but few exceptions. Contrary to the results of Tagawa, glutamic acid hydrochloride was found in our work to be the weakest instead of the strongest stimulant for gastric secretion. All of the free acids, with the exception of isoleucine, were found to be slightly more active than glutamic acid hydrochloride. The fact that glutamic acid was a more potent stimulus for secretion than its hydrochloride is directly opposite to the findings of Tagawa.

Isoleucine, arginine and histidine were found to produce from 2 to 3 times the volume of juice excited by the other amino-acids tested. The activity of histidine in this respect is particularly interesting in view of its close structural relationship to histamine. The potency of isoleucine argues against the view that the basicity

* These data form a part of the dissertation submitted by Elizabeth R. B. Smith to the Faculty of the Graduate School, Yale University, May, 1933, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[1] Tagawa, J., *Biochem. Z.*, 1931, **243**, 344.

[2] Ivy, A. C., and Javois, A. J., *Am. J. Physiol.*, 1924, **72**, 591.